cancellation of the claim. The concerns raised by the Examiner are addressed below as set forth in the Final Action.

I. Claim Rejections Maintained Under 35 U.S.C. § 102

The Final Action maintains the rejection of claims 16 and 31-33 under 35 U.S.C. § 102(b) as allegedly being anticipated by Silvestris et al. *Ann Hematol.* **70(6)**: 313-318 (1995) (hereinafter, "Silvestris et al.") for the reasons of record.[1] The Final Action further states that "Silvestris et al. still discloses a method of administering erythropoietin (epo) within the dose ranges that would help treat endothelial cell injury. Although it is not implicitly stated, the endothelial cells would be damaged because as stated in the specification and claimed, cancer is a cause for said endothelial cell injury." Final Action, pages 2-3. Applicants respectfully disagree with this assertion.

As noted by the Federal Circuit, "[a]nticipation under 35 U.S.C. § 102 requires the disclosure in a single piece of prior art of each and every limitation of a claimed invention." *Apple Computer Inc. v. Articulate Systems Inc.* 57 USPQ2d 1057, 1061 (Fed. Cir. 2000) (*relying on Electro Med. Sys. S.A. v. Cooper Life Scis.*, 32 USPQ2d 1017, 1019 (Fed Cir. 1994). A finding of anticipation further requires that there must be no difference between the claimed invention and the disclosure of the cited reference as viewed by one of ordinary skill in the art. *See Scripps Clinic & Research Foundation v. Genentech Inc.*, 927 F.2d 1565, 1576, 18 U.S.P.Q.2d 1001, 1010 (Fed. Cir. 1991). Additionally, the cited prior art reference must be enabling, thereby placing the allegedly disclosed matter in the possession of the public. *In re Brown*, 329 F.2d 1006, 1011, 141 U.S.P.Q. 245, 249 (C.C.P.A. 1964). Thus, the prior art reference must adequately describe the claimed invention so that a person of ordinary skill in the art could make and use the invention.

Applicants respectfully submit that Silvestris et al. fails to disclose each and every recitation of claims 16 and 31-33 and fails to enable one of ordinary skill in the art to make and use the invention as recited in claims 16 and 31-33. More specifically, claim 16 recites as follows:

> 16. A method of treating **endothelial injury** in a subject, comprising administering an effective **endothelial-protecting amount** of erythropoietin to said subject in need of such treatment,

[1] The Examiner cited the abstract for Silvestris et al. For the Examiner's convenience, Applicants provide herewith the complete reference.

> wherein said **endothelial injury** is caused by mechanical damage, exposure to radiation, inflammation, heart disease or cancer. (Emphasis added).

As noted in the present application, at page 2, lines 4-12, the vascular endothelium is a layer of cells lining the inner vascular wall and in direct contact with blood, providing an active natural barrier between the circulatory and extravascular compartment. The endothelium is involved in signal and information transfer at the cellular, tissue and organ level, and plays a role in both cell-mediated and humoral immune responses. Endothelial cells are metabolically active and normally produce a number of substances with effects on the vascular lumen and on platelets. Endothelial cells also secrete growth factors which enhance endothelial mitogenesis and can induce new blood vessel formation (angiogenesis).

Silvestris et al. is directed to studying the effectiveness of recombinant human erythropoietin (rHu-EPO) in the treatment of **anemia** associated with progressing multiple myeloma. *See* Silvestris et al. Referring to *Webster's Third New International Dictionary*, anemia is defined as "a condition in which the blood is deficient in red blood cells, hemoglobin, or both or deficient in total volume." As noted above, the endothelium pertains to the layer of cells lining the inner vascular wall and vascularization, in general. Anemia is clearly not a function of vascularity. Thus, the treatment proposed by Silvestris et al. is directed to an entirely different physiological condition than a method of treating endothelial injury as disclosed in the present application and as recited in claims 16 and 31-33. Consequently, Silvestris et al. fails to disclose each and every recitation of claim 16 and 31-33 and fails to provide an enabling disclosure that would enable one of ordinary skill in the art to make and use the invention as recited in claims 16 and 31-33. It is only through impermissible hindsight employing the teachings of the present application is one of ordinary skill in the art able to arrive at the present invention directed to methods of treating **endothelial injury**. Accordingly, Applicants respectfully request that the rejection of claims 16 and 31-33 under 35 U.S.C. § 102(b) be withdrawn.

II. New Claim Rejections Under 35 U.S.C. § 102

A. Claims 16, 18, 21, 22, 31, 32, 35 and 40 stand rejected under 35 U.S.C. § 102(b) as being anticipated by JP 02 096535 to Chugai Pharm Co. Ltd. (hereinafter, "JP 02 096535). More specifically, the Final Action alleges that "[b]ecause the method of the prior

art [JP 02 096535] comprises the same method steps claimed in the instant invention, that is, administering EPO for the treatment of disease caused by the same mechanism (i.e., exposure to radiation or as a result of the treatment of cancer by carcinogenic compounds such as cisplatin), the claimed method is anticipated because the method will inherently treat endothelial cells that are damaged as a result of the aforementioned mechanisms of disease." Final Action, page 3. Applicants respectfully disagree with this assertion.

As noted above in Section I., "[a]nticipation under 35 U.S.C. § 102 requires the disclosure in a single piece of prior art of each and every limitation of a claimed invention." *Apple Computer Inc*, 57 USPQ2d at 1061. In this instance, JP 02 096535 is directed to "therapy of anaemia due to radiation exposure or administration of carcinostatic substance." *See* abstract. Thus, this reference is also directed to the treatment of **anemia** and not treatment of endothelial cell injury as recited in the claims of the present invention. However, the Final Action alleges that the claimed method is anticipated because the method will inherently treat endothelial cells that are damaged as a result of the aforementioned mechanisms of disease.

The legal standard for inherency, as set forth in the Manual of Examining Procedure (hereinafter, "M.P.E.P.") § 2112, states that "[t]o establish inherency, the extrinsic evidence 'must make clear that the missing descriptive matter is necessarily present in the thing described in the reference, and that it would be so recognized by persons of ordinary skill. The M.P.E.P. also cites *Ex parte Levy* as stating that "[i]n relying upon the theory of inherency, the examiner must provide a basis in fact and/or technical reasoning to reasonably support the determination that the allegedly inherent characteristic necessarily flows from the teachings of the applied prior art. (17 USPQ2d 1461, 1464 (Bd. Pat. App. & Inter. 1990)). It is clear from these cases that in order for the standard for inherency to be properly cited in an anticipation rejection, the allegedly inherent characteristic must necessarily flow **from the teachings of the cited art** and such a determination must be supported by fact or technical reasoning. In this instance, such teachings do not flow from the teachings of the cited reference. Again, it is only through impermissible hindsight employing the teachings of the present application is one of ordinary skill in the art able to arrive at the present invention directed to methods of treating **endothelial injury**. Accordingly, Applicants respectfully request that the rejection of claims 16, 18, 21, 22, 31, 32, 35 and 40 under 35 U.S.C. § 102(b) be withdrawn.

B. Claims 16, 21, 31, 32, 33 and 35-38 stand rejected under 35 U.S.C. § 102(b) as being allegedly anticipated by Bukowski et al. *Blood* **84 (10 Supp. 1):** 129A (1994) (hereinafter, "Bukowski et al."). The Final Action alleges that "because the method of the prior art [Bukowski et al.] comprises the same method steps as claimed in the instant invention, that is, administering EPO in conjunction or concomitantly with cisplatin within the same endothelial-inhibiting dosage range, the claimed method is anticipated because the method will inherently treat the injured endothelial cells." Final Action, page 3. Applicants respectfully disagree with this assertion.

Bukowski et al. is directed to evaluation of clinical outcomes of anemic cancer chemotherapy patients receiving EPO. *See* abstract. Treatment of **endothelial injury** does not flow from the teachings of the cited reference. Yet again, it is only through impermissible hindsight employing the teachings of the present application is one of ordinary skill in the art able to arrive at the present invention directed to methods of treating **endothelial injury**. Accordingly, Applicants respectfully request that the rejection of claims 16, 18, 21, 22, 31, 32, 35 and 40 under 35 U.S.C. § 102(b) be withdrawn.

In summary, use of EPO for *(a)* treatment of a patient population suffering from anemia caused from bone marrow function (as suggested by JP 02 096535) or *(b)* treatment of a patient population suffering from anemia to improve clinical outcomes (as suggested by Bukowski et al.) does not provide an adequate disclosure for treatment of endothelial injury.

Accordingly, Applicants respectfully request that the rejection of claims 16, 18, 21, 22, 31, 32, 35 and 40 in view of JP 02 096535 and claims 6, 21, 31, 32, 33 and 35-38 in view of Bukowski et al. under 35 U.S.C. § 102(b) be withdrawn.

III. Claim Objections

Claims 17 and 19-20 stand objected to as being dependent upon rejected base claim 16. For at least the reasons set forth above, Applicants respectfully submit that claim 16 is patentable. Accordingly, Applicants respectfully request that this objection be withdrawn.

Conclusion

In view of the foregoing remarks, Applicants respectfully request that all outstanding rejections and objections to the claims be withdrawn and that a Notice of Allowance be issued in due course. The Examiner is invited and encouraged to contact the undersigned

directly if such contact will expedite the prosecution of the pending claims to issue. In any event, any questions that the Examiner may have should be directed to the undersigned, who may be reached at (919) 854-1400.

It is not believed that any fee(s), including fees for additional claims, are required, beyond those that may otherwise be provided for in documents accompanying this paper. In the event, however, that additional fees are necessary to allow consideration of this paper, such an extension is also hereby petitioned for under 37 C.F.R. §1.136(a). Any additional fees believed to be due in connection with this paper may be charged to our Deposit Account No. 50-0220.

Respectfully submitted,

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ORIGINAL ARTICLE

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Long-term therapy with recombinant human erythropoietin (rHu-EPO) in progressing multiple myeloma

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Abstract Recombinant human erythropoietin (rHu-EPO) is an effective growth factor for erythroid progenitor cells in anemia provoked by several conditions, including bone marrow tumors such as multiple myeloma (MM). We studied a group of 54 patients with MM undergoing second-induction chemotherapy. Thirty of them were randomly assigned to receive rHu-EPO at an initial dosage of 150 units/kg body weight three times a week, increased to 300 units/kg from the sixth week to the end of the 24-week study. Hemoglobin (Hb) levels increased in 77.7% of these patients by the eighth week. In addition, five transfusion-dependent patients in treatment with the VMCP protocol completed the trial without requiring blood supplement after the third month, whereas seven control patients required frequent supplements. Monthly assessment of hematologic parameters demonstrated the ability of rHu-EPO to increase reticulocyte counts, whereas five patients became resistant to the second-induction chemotherapy in apparent concurrence with their rHu-EPO therapy. The response to rHu-EPO in four of the five MM patients receiving cytotoxic protocols combined with α-interferon (α-IFN) included an increase of serum IgM after the third month. This effect was not demonstrable in any other group, including three rHu-EPO-untreated patients undergoing α-IFN + VMCP combined therapy, as well as rHu-EPO-treated patients not receiving α-IFN. Our data suggest that α-IFN plus rHu-EPO treatment in MM patients is effective in restoring normal B cell function. These results may reflect in vivo the modulation of normal human B cells and lymphoblasts by rHu-EPO observed in vitro.

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Key words Anemia · α-Interferon · Chemotherapy
Multiple myeloma · rHu-EPO

Introduction

Recombinant human erythropoietin (rHu-EPO) is a pleiotropic growth factor acting on different hematopoietic progenitor cells [3, 12] which has been shown to correct anemia associated with chronic renal failure [6, 8, 15, 16]. Although rHu-EPO therapy does not significantly influence leukocyte or platelet counts, a generalized increase in both megakaryocyte proliferation and the number of marrow colony-forming cells has been reported [4, 34]. Furthermore, an immunomodulatory effect on both T-cell activation and B-cell proliferation has been detected in vivo in chronically dialyzed patients undergoing long-term rHu-EPO treatment [16].

The efficacy of rHu-EPO is being explored in other situations, in addition to chronic renal diseases. Patients with solid tumors and myelodysplasia have lower EPO levels than their degree of anemia would suggest [17, 25], whereas the expected linear relation between serum EPO and Hb is usually absent, and the EPO response may be further depressed during chemotherapy [30].

rHu-EPO improves the hematocrit in malignant lymphoma [28] and myelodysplastic syndromes [11, 32, 35] and has corrected the anemia of progressing multiple myeloma (MM) [23, 29]. The response to rHu-EPO is not apparently dependent on pretreatment serum EPO levels, which are not correlated with the severity of anemia. However, the cytotoxic protocols adopted to treat relapsing MM may be a major cause of erythropoiesis suppression [31], which regularly aggravates the chronic bone marrow erythroid deficiency.

Treatment of resistant MM with α-interferon (α-IFN) alone has produced responses in about 14%

of patients [7, 9], whereas its combination with high-dose prednisone [36] or cytotoxic agents [33] results in higher and quicker response rates. No data are available on the concurrent use of recombinant erythropoietic factors and α-IFN in MM.

Since α-IFN inhibits B-cell lineage differentiation, combined α-IFN plus rHu-EPO therapy of resistant MM may be of interest in restoring both erythropoiesis and normal T- and B-cell immune function. This paper presents the results of a long-term trial using rHu-EPO in MM patients undergoing second-induction chemotherapy. Combined treatment with α-IFN was also attempted in several instances. Our data support the contention that rHu-EPO modulates several immune functions in progressing MM, in addition to improving bone marrow erythroid regeneration.

Materials and methods

Patients

Fifty-four patients with MM stages I–III A [14], resistant to conventional melphalan-prednisone (MP) chemotherapy, were included in this study. They satisfied the following criteria: (a) chronic anemia (Hb level $\leq$ 8.0 g/dl) with or without transfusional supplementation; (b) commencement of second-induction chemotherapy; (c) preserved kidney function; (d) Karnofsky performance status lower than 50 [20]. They were classed as transfusion-dependent (TD) or not transfusion-dependent (NTD) according to their requirements of one or more instances of red cell unit supplementation within the 3 months preceding enrollment. Eight patients had been receiving α-IFN (9 MU/week) since reaching complete remission. Patients were randomly assigned to the rHu-EPO-treated (EPO^+) or -untreated (EPO^-) arms. Randomization of the patients was obtained directly by the biostatistical department of the pharmaceutical company providing the recombinant hormone (Cilag AG, Schaffhausen, Switzerland). Informed consent was obtained and the study was approved by the Ethical Committee of the University of Bari.

rHu-EPO-treatment

Thirty patients were given an initial dose of 150 units/kg of rHu-EPO (Cilag AG) subcutaneously three times a week. This dose was increased to 300 units/kg by the sixth week of treatment. In most instances, rHu-EPO was administered on an outpatient basis for 24 weeks. An increase of 1.2 m*M* (2 g/dl) or more of the initial Hb level [23] or, alternatively, no further red cell supplementation in TD patients was arbitrarily taken as response to the treatment. Regular iron administration was provided throughout the study.

Combination therapies

The rHu-EPO treatment was started within the first month of conventional cytotoxic protocols [1] including VMCP (group A: vincristine plus melphalan plus cyclophosphamide plus prednisone; 37 patients); VMCP plus α-IFN (group B; eight patients); VED (group C: vincristine plus epirubicin plus dexamethasone; seven patients), or CTX (group D: high-dose cyclophosphamide; two patients).

Clinical and laboratory evaluations

Weekly controls included a thorough physical examination, a complete blood count including red cell, reticulocyte, leukocyte differential, and platelet counts, Hb and hematocrit levels, iron, transferrin and ferritin concentrations, electrolytes, and kidney function tests. Physical evaluation also included monthly assessment of the performance status. Total serum levels of normal Ig and the monoclonal component, as well as Bence-Jones proteinuria, were monitored monthly on the occasion of the scheduled cytotoxic protocols.

Statistical evaluations

Since the majority of the laboratory parameters studied are not normally distributed, ANOVA was performed by evaluating the median of each parameter and its range between minimum and maximum. The Wilcoxon test was adopted as a nonparametric method to compare different groups.

Results

Table 1 shows the distribution of patients grouped by chemotherapy protocols, rHu-EPO treatment, and transfusional regimen. A response ($\geq$ 2 g/dl increase in Hb concentration) was observed in 21 of 27 (77.7%) evaluable patients after a median period of 8 weeks, although in three instances (nos. 25, 40 and 49) it occurred during the third month, in one (no. 15) after the fourth. rHu-EPO was well tolerated, and mild hypertension was recorded in only four cases. The first of four drop-out patients (nos. 13, 33, 42, and 45) suffered a cerebral vascular stroke during the fifth week. Patient 33 was lost to follow-up at the seventh week, whereas the remaining two patients were excluded at the third and the 11th week because of severe pneumonia and multiple bone fractures, respectively. No evident Hb increase was observed in the three EPO^+ drop-out patients during their inclusion in the trial.

The Hb variations are shown in Fig. 1. A remarkable increase and stabilized levels were observed after 2 months (section a) in NTD-EPO^+ patients undergoing the VMCP regimen (group A). The highest median value was detected in nine responders during their 12th week of treatment, showing a significant difference ($p < 0.05$) as compared with the initial median value. Conversely, median values in the 11 NTD-EPO^- controls from group A were generally stable during the trial. Fig. 1b illustrates the Hb profile of the TD patients from group A. As can be seen, the Hb changes in the five EPO^+ patients were lower than those in the NTD group. However, substantial maintenance of restored Hb levels was observed in four of the five EPO^+ patients, in that they no longer required blood transfusions after 8 weeks of rHu-EPO therapy. In contrast, the four EPO^- TD controls from group A displayed a lesser degree of Hb improvement as compared with EPO^+ patients, although they received continuous transfusional supplementation. Five EPO^+

Table 1 Distribution of 54 myeloma patients enrolled into the trial, grouped by their chemotherapy protocols, transfusion dependency, and response to the recombinant erythropoietin treatment (*NTD* non-transfusion dependent, *TD* transfusion dependent)

Chemotherapy groups	r-Hu-EPO-treated patients				Control patients	
	NTD (*n*)	Responders evaluable patients	TD (*n*)	Responders evaluable patients	NTD (*n*)	TD (*n*)
A VMCP	11	9/10	9	5/8	12[a]	5[b]
B VMCP + α-IFN	5	5/5	–	–	3	–
C VED	1	–	3	2/3	1	2
D CTX	–	–	1	–/1	–	1

[a] Eleven patients were evaluable at the end of the study.
[b] Four patients were evaluable at the end of the study.

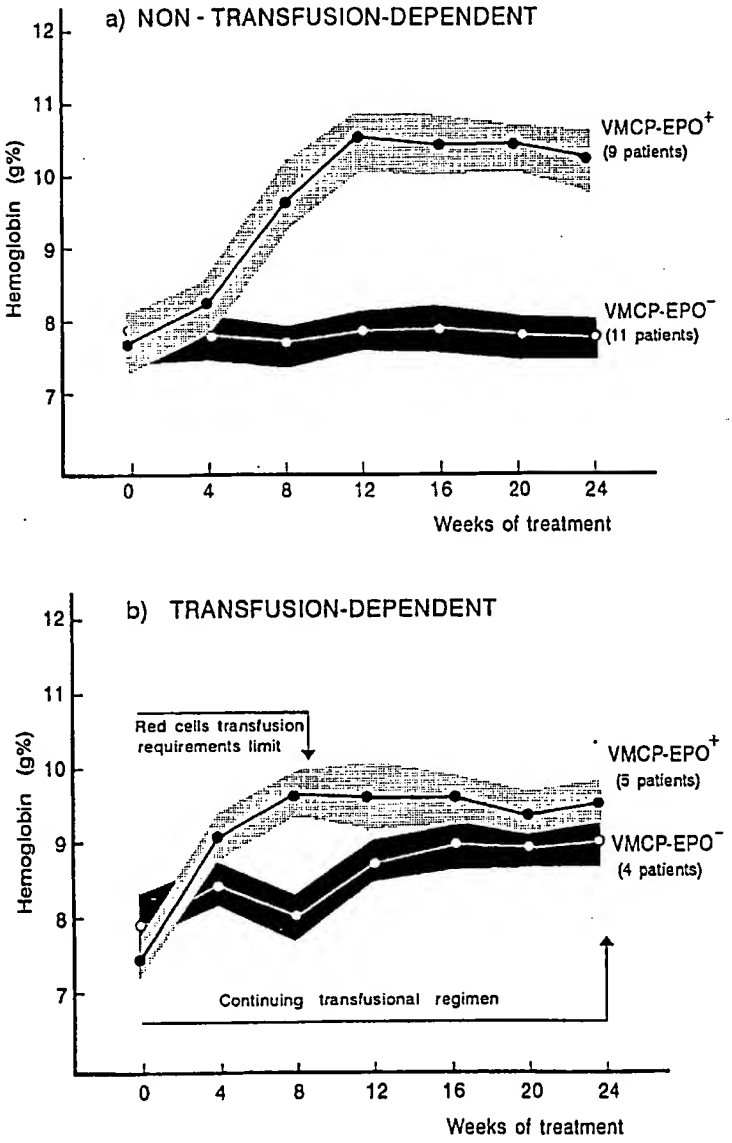

Fig. 1a, b Effect of rHu-EPO on Hb levels in 14 patients with progressing multiple myeloma (MM) receiving VMCP and divided into two groups (a and b) according to function of their transfusion requirements. In NTD patients, rHu-EPO promoted a significant ($p < 0.05$) and stable increase in Hb levels by the 12th week as compared with initial values. This enhancement was lower in five responder TD patients from group (b) who completed the 24-week trial without requiring blood supplements after the fourth month. Results are expressed as median values of Hb levels of each group, including minimum and maximum. *Arrows* indicate the period of transfusional needs

TD patients were nonresponders, although two nos. (31 and 41) now required fewer transfusions.

Mild to severe leukopenia and/or thrombocytopenia was detected in most patients in relation to the cytotoxic protocols they were given, without a significant difference between the EPO^+-treated patients and controls. Figure 2 shows the effect of the chemotherapy regimens on the peripheral reticulocyte counts of the NTD patients. A slight although significant ($p < 0.05$) increase of the median value during the fourth month of rHu-EPO therapy reflected the augmentation of median Hb levels, although a slight decline was recorded in the following weeks. Transferrin and ferritin serum levels were not significantly influenced as the trial proceeded.

Monthly monitoring of the monoclonal components revealed a general pattern of stability or reduction of myeloma proteins related to continuation of the cytotoxic treatment. However, short-lived responses to chemotherapy or progressing disease were observed in eight patients. Some of them required the adoption of further combined protocols during the trial. The relationship of such relapsing diseases to the rHu-EPO treatment is shown in Table 2. In two of these patients (nos. 15 and 36) a serum increase of the myeloma protein was noted in the second month of chemotherapy, in apparent concurrence with the increment of the rHu-EPO dose.

Normal Ig isotypes were also monitored in all patients during the trial. Their levels were uniformly low and stable in the EPO^- patients until the end of the study. Conversely, variable fluctuations of serum IgM were revealed in several rHu-EPO responders (Fig. 3). A significant increase ($p < 0.05$) of this isotype was observed after the third month of therapy in four patients (nos. 5, 37, 43, and 47) from group B (VMCP regimen plus α-IFN). Sixteen responder patients (14 from group A and two from group C) used as controls did not display elevations of IgM similar to those detected in the four patients receiving rHu-EPO plus α-IFN. Finally, serum polyclonal IgA and IgG levels were persistently low with slight modifications throughout the trial in all patients of groups A, B, and C, whether they received rHu-EPO or not.

Table 2 Variations of monoclonal component (*MC*) levels in eight myeloma patients resistant to second-induction chemotherapy. EPO^+ patients 15 and 36 manifested elevations of their MC levels during the third month of therapy. (*Arrows* indicate the time of administration of further cytotoxic protocols)

Patient number	Chemotherapy	rHu-EPO random assignment	Levels of MC (g%)		
			Month of treatment		
			2nd	4th	6th
15	VED	EPO^+	2.8	6.4	6.1
19	VMCP/VED	EPO^+	3.2	4.1	5.9
23	VMCP	EPO^-	3.4	5.8	6.2
24	VMCP	EPO^-	2.6	3.2	2.9
36	VMCP/VED	EPO^+	3.5 →	8.8	4.0
38	VMCP	EPO^+	2.8	3.0	3.4[a]
44	VED/DMS	EPO^-	2.2	3.0 →	5.1
51	VMCP	EPO^+	2.1	2.8	2.4

[a] BJk proteinuria/24h

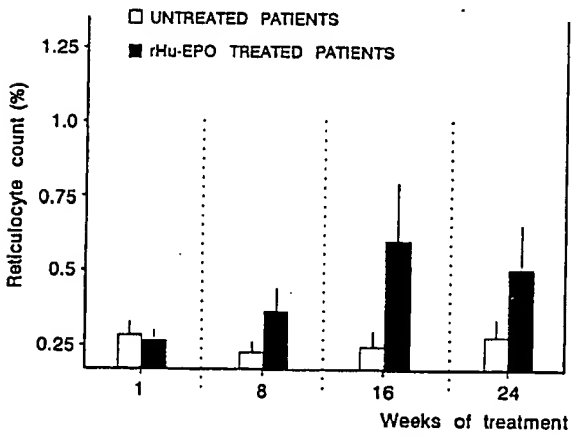

Fig. 2 Changes of median reticulocyte counts in patients with MM following second-induction chemotherapy in comparison to patients undergoing their cytotoxic protocols combined with the 24-week rHu-EPO therapy

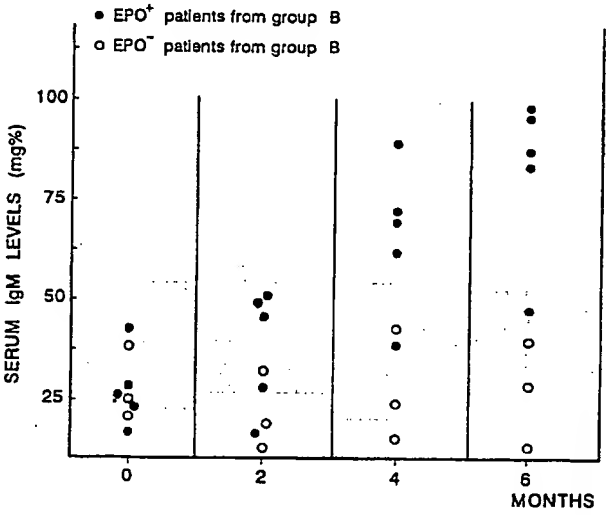

Fig. 3 Individual serum IgM levels of five rHu-EPO-treated patients in combination with α-IFN (9 MU/week) as compared with three rHu-EPO-untreated controls. Significant elevations ($p < 0.05$) were observed in four of the five patients receiving both cytokines by the fourth month of their treatment. In contrast, no evident variations of circulating IgM were noted in three α-IFN-treated patients not receiving the rHu-EPO. *Shaded areas* represent the median values, including minimum and maximum of serum IgM levels detected in 14 and two responder patients from groups A and C, respectively, used as α-IFN-untreated controls

Discussion

This study provides further evidence of the effectiveness of rHu-EPO in the treatment of anemia associated with progressing MM [5, 23, 29]. Most MM patients (77.7%) were highly responsive to rHu-EPO, and their Hb levels were consistently elevated throughout the trial. In addition, five VMCP-treated patients and one VED-treated patient previously requiring periodical transfusions completed the trial and needed no more transfusions after the second month. Five NTD patients on VMCP plus α-IFN responded to rHu-EPO, and four of them displayed an increase of serum IgM. The therapy was safe and substantially devoid of side effects. Moreover, general well-being was reported by several responders.

A few questions remain with regard to six nonresponders, including both TD and NTD patients. In a previous study [23] no correlation was shown between serum endogenous EPO levels and response to rHu-EPO treatment, concerning the clinical and morphological features of erythroid progenitor regeneration. The replacement of normal progenitors by malignant plasma cells has been postulated as a major cause of marrow unresponsiveness to rHu-EPO in selected MM patients [2]. However, the impaired hematopoietic response to rHu-EPO is often related to the inadequate supply of iron, as well as to concurrent bacterial infections or inflammation [19, 26]. Since patients were regularly given appropriate amounts of iron and no evident infections occurred during the trial, failure of rHu-EPO therapy in a relatively small proportion of MM patients may be tentatively ascribed to one or more of the following factors: (a) poor cellular expression of EPO receptors [19]; (b) MM-related suppression of erythropoiesis; (c) erythron-based hyporesponsiveness following its regenerative defect; (d) immunization of patients to the rHu-EPO.

A second point of interest is the occurrence in five patients (nos. 15, 19, 36, 38, and 51) of an increase of their myeloma proteins following rHu-EPO treatment. Promotion by EPO of the proliferation of normal plasma cells [22] and enhancement of their Ig secretion [18] has been observed in vitro. However, although an aberrant expression of EPO receptors has been reported by Okuno et al. [27] on a human in vitro stabilized myeloma cell line, to our knowledge, no further studies have been presented to support a general expression of EPO receptors on different myeloma cell lines. In addition, the secretive effect of rHu-EPO on human malignant plasma cells in vitro has not yet been demonstrated. Thus, tumor relapse, rather than in vivo response to the cytokine, is the most likely explanation for the increased secretion of myeloma proteins in these patients [24]. On the other hand, the response to EPO treatment in three patients with relapsing MM (nos. 15, 36, and 38) suggested that the efficacy of rHu-EPO in responder patients was poorly or not at all related to the beneficial effect of the chemotherapy regimen.

One striking result of our work was the unexpected modulation of certain immune functions in MM when rHu-EPO was used in combination with a biological response modifier such as α-IFN. Although only five patients received such a combined therapy, a significant increase ($p < 0.05$) of serum IgM levels was observed in four of them with the progression of the trial. This effect was apparently related to the concurrent administration of both cytokines, since in three patients from group B receiving α-IFN only and in 14 EPO^+ patients from group A, no significant IgM fluctuations were noted. However, normal B-cell function is persistently depressed in patients with progressing MM, who regularly display low circulating levels of nonmyelomatous polyclonal Ig. Thus, treatment with rHu-EPO plus α-IFN may reflect in vivo the modulating effect on some immune functions shown in vitro. In addition, changes of serum IgM levels in these patients were not paralleled by an increase of their myeloma proteins, which remained stable (no. 37) or showed a progressive reduction by the third to fifth month (nos. 5, 43, 47) in relation to the chemotherapy regimen.

The significant increase of serum IgM levels in four α-IFN + rHu-EPO-treated patients obviously needs confirmation in a much larger number of cases. However, it appears to support the stimulatory activity of rHu-EPO on human B cells [16]. In this context, Kimata et al. [21, 22] showed that human tonsil B cells and CLB lymphoblasts supplemented in vitro with 5 units/ml of rHu-EPO significantly increased the production of IgA and IgM as well as their thymidine uptake. Since α-IFN is suspected to arrest the maturation of malignant plasma cells at the G0-G1 level, as in melanoma cells [10], the tendency of normal, α-IFN-inhibited B cells to improve Ig secretion in our patients may result from restoration of their function induced by the rHu-EPO. Also, it may be that the improvement of erythropoiesis in patients receiving rHu-EPO might somehow enhance their immune responsiveness.

Additional work is required to define the biological effects of rHu-EPO on the immune system and establish the occurrence and significance of EPO receptors on B cells. The potential expression of these receptors on malignant plasma cells could increase the risk of activating their growth during rHu-EPO therapy.

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